Significance of hypocalcemia in predicting dengue severity in the pediatric population: A systematic review and meta-analysis

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Abstract

Introduction Dengue is one of the causes of morbidity and mortality among pediatric patients. Calcium has been shown to play a role in the myocardial function of the patient and is a potential cause of mortality among dengue patients. This study aimed to determine the accuracy of hypocalcemia in predicting the severity of dengue among pediatric patients.

Methods This is a systematic review and meta-analysis of published studies identified through an electronic literature search using PubMed/Medline, Cochrane Library, Herdin, Google Scholar, and hand search. Validity was assessed using the Cochrane risk of bias tool. Statistical analysis of the diagnostic test accuracy review was done using Review Manager 5.4.1 with the random effects model. Results showed sensitivity and specificity of hypocalcemia in severe dengue with a 95% confidence interval. The predictive values and likelihood ratios were also computed.

Results Four studies were analyzed. The mean serum total and ionized calcium levels of patients were decreased among the severe dengue group. Data showed that there is a 74% sensitivity (95% CI = 0.58, 0.84) and 75% specificity (95% CI = 0.67, 0.81) with a positive predictive value of 67% and a negative predictive value of 90.7%.

Conclusion This systematic review and meta-analysis showed that hypocalcemia may be a useful tool to predict severe dengue fever. However, further analysis is needed to strengthen the the diagnostic accuracy of hypocalcemia.

Key words: hypocalcemia, severe dengue, meta-analysis

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Dengue is very common in the Philippines especially during rainy season. It has been one of the morbidity cases seen in hospitals and can be a part of the mortality census. Mortality among pediatric patients can be prevented with adequate hydration and recognizing complications early in the course of the illness. There are different studies that focus on determining electrolyte imbalances seen during the course of the disease and calcium is the main interest of the researchers. Calcium has been shown to play a

role in the myocardial function of the patient and a low level is a potential cause of mortality.

Dengue is a mosquito-borne viral disease that is transmitted by female *Aedes aegypti* mosquitoes. It has rapidly spread in all regions in recent years. It is widespread throughout the tropics including the Philippines where there is a mixture of heavy rainfall and high relative humidity.[1] Dengue has four distinct serotypes which are DENV-1, DENV-2, DENV-3 and DENV-4. Recovery from the infection is believed to provide lifelong immunity against that serotype. Subsequent infections (secondary infection) by other serotypes increase the risk of developing severe dengue.

There is an estimate of 390 million dengue infections per year. The number of dengue cases reported has increased more than eight-fold over the last two decades from 505,430 cases in 2000 to 4.2 million in 2019. Dengue fever can range from a subclinical disease to severe flu-like symptoms. Some patients develop severe dengue with a number of complications like severe bleeding, organ impairment and/or plasma leakage. Severe dengue has a higher risk of death when not managed appropriately. As such, reported deaths from 2000 and 2015 increased from 960 to 4,032.[1]

The World Health Organization (WHO) classifies dengue into two major categories: dengue (with/without warning signs) and severe dengue.[1] Dengue should be suspected when a high grade fever is accompanied by any two of the following symptoms: severe headache, pain behind the eyes, muscle and joint pain, nausea, vomiting, swollen glands and rash. Severe dengue is diagnosed when the patient enters the "critical phase" and presents with signs of severe plasma leakage, fluid accumulation, respiratory distress, severe bleeding or organ impairment. Complications from severe dengue increase the risk of mortality. The dengue virus may be isolated from the blood during the first few days of infection and can be detected by testing for a virus-produced protein called NS1. Another test is the enzyme-linked immunosorbent assay (ELISA) that may confirm the presence of a recent or past in infection with the detection of IgM and IgG antibodies.

In severe dengue infection, there are several serum biochemical parameter changes that occur with the onset of plasma leakage such as decreased levels of calcium.[2] In some studies, hypocalcemia has been documented in dengue infection and is seen frequently among severe dengue patients.[3] Shivanthan and Rajapakse noted that the depletion of magnesium and calcium has been shown to enhance binding of dengue virus to monocyte macrophages and cells of T-cell and B-cell lineages.[3] Calcium is essential for the cytotoxic activity of dengue virus and this increased intracellular calcium leads to cell death. Calcium also has a role in the induction of dengue-specific T-helper cells; the dengue antigen increases the influx of calcium into the T-cells, hence leading to further cell death. Dahanayaka postulated that hypocalcemia in dengue fever could be due to influx of calcium and calcium replacement could enhance the dengue virus activity by increasing intracellular calcium ion concentration.[4]

Calcium plays a role in platelet aggregation and immune response in dengue infection.[5] Patients with hypocalcemia may present with hypotension, reduced myocardial function, electrocardiogram (ECG) abnormalities and heart failure which may worsen the patient's condition and lead to death. Since calcium plays a role in the functioning of myocardial tissue, derangements of calcium levels may directly contribute to myocarditis. Patients present with ECG changes like sinus bradycardia, tachycardia and T-wave inversion, and elevated creatine phosphokinase-MB (CPK-MB) levels.

In some studies, calcium levels were determined by testing either for the total calcium or ionized calcium. These studies revealed varied results with regard to the severity of dengue. Jayachandra stated that the calcium ion plays a critical role in normal cellular function and signaling, regulating diverse physiologic processes such as neuromuscular signaling, cardiac contractility, hormone secretion, and blood coagulation.[6]

Total serum calcium exists in three forms: 1) ionized, normally 50% of the total; 2) bound to plasma proteins such as albumin, usually 40% of the total; and 3) complexed to anions such as lactate and phosphate, usually 10% of the total. Ionized calcium, the physiologically active form of calcium found in the blood, is regulated by homeostasis. The total calcium level, therefore, is influenced directly by the serum albumin concentration. Free calcium is a more useful index than total calcium and provides a better indication of calcium status.

There are few literature documenting the role serum calcium levels in dengue. Some studies showed that dengue severity is correlated with the serum

ionized calcium levels. The general objective of this study is to determine the accuracy of hypocalcemia in predicting dengue severity among the pediatric age group. The specific objectives are to: a) determine the serum calcium levels of the dengue patients and b) compare the serum calcium levels of the pediatric patients diagnosed with non-severe dengue (dengue with and without warning signs) and severe dengue.

Methods

This study is a systematic review and meta-analysis of published studies that fulfill the inclusion criteria. Electronic literature search using PubMed/Medline, Cochrane Library, Herdin databases, Google Scholar and hand search was done using the following key words: severe dengue, hypocalcemia, low serum calcium level and children. Search was limited to descriptive studies on patients aged 0-18 years old, published until the time of study. Abstracts, single case reports and letters were not included in the analysis but were used as supporting literature. Bibliographies of studies and related articles were also scanned to identify additional trials and other relevant publications.

A study was included in this review if the following criteria were met: a) serum calcium levels of patients was tested and correlated with dengue fever; b) patients were aged 0-18 years; c) diagnosis and confirmation of dengue through Dengue NS1 and/or Dengue IgG, IgM and/or ELISA rt-PCR; d) severity of dengue of the patients was specified; and e) serum calcium levels of the patients were stated. A study was excluded in this review if any the following criteria were present: a) unavailability of full text; and b) patients received calcium supplement.

Once the studies were adequately screened, the authors independently appraised the validity and applicability based on the user's guides in Table 1. The studies for screening were also assessed for the risk of bias using the Cochrane risk of bias tool. Each criterion was scored as "low risk", "high risk" and "unknown risk" signifying potential for risk of bias in the respective category. The authors scored the studies independently and any disagreements were resolved with the aid of the third party.

Statistical analysis was done by recording the data into the Review Manager 5.4.1 program. A random effects model was used. The author was guided through the Cochrane diagnostic test accuracy review which provided information on how well the test distinguished patients with the disease from those without. The statistical analysis included the sensitivity of the test, which tells the proportion of those with severe dengue who have hypocalcemia, and the specificity of the test, which tells the proportion of those with non-severe dengue (dengue with or without warning signs) who have a normal calcium level. Additional computations specifically the manual computation of the sensitivity and specificity were done by making a 2 x 2 cross classification table of hypocalcemia and severe dengue. The results of the sensitivity and specificity of the included studies were plotted in the forest plot and receiver operating characteristic (ROC) curve. The positive (PPV) and negative predictive values (NPV), likelihood ratios (LR+, LR-) and diagnostic odds ratios were also computed.

Table 1. User's guides for appraising validity, results and applicability

1. Validity
   a. Was there an independent blind comparison with a reference standard?
   b. Did the patient sample include an appropriate spectrum of patients to whom the diagnostic test will be applied in clinical practice?
   c. Did the results of the test being evaluated influence the decision to perform the reference standard?
   d. Were the methods for performing the test described in sufficient detail to permit replication?

2. Results
   a. Are likelihood ratios for the test results presented or data necessary for their calculation provided?

3. Applicability
   a. Will the reproducibility of the test result and its interpretation be satisfactory in my setting?
   b. Are the results applicable to my patient?
   c. Will the results change my management?
   d. Will patients be better off as a result of the test?

Results

A total of 28 studies were identified in the initial search. Four studies were added with the hand search. After removing duplicates, 32 studies were screened. Five studies were excluded due to the inability to acquire the full text and 13 studies were excluded due to the irrelevance of the title and abstracts. The remaining 14 articles were then screened for eligibility based on the inclusion criteria and objectives of the study. Ten studies were excluded for incomplete data and risk for bias. In the 10 excluded studies, categorization of dengue was not specified, or no mean result of the ionized calcium was provided. The identification of studies was summarized in the PRISMA flow diagram (Figure 1). Four articles (Kumar 2017, Manjunath 2019, Nguyen 2004, Singh 2019) were included in the systematic review and meta-analysis and their study characteristics are shown in Table 2.[5,7-9]

Validity of included studies was assessed using the Cochrane risk of bias tool. All the included studies had low risk of bias on patient selection, index test, reference standard and flow and timing. However, one study showed unclear applicability in the patient

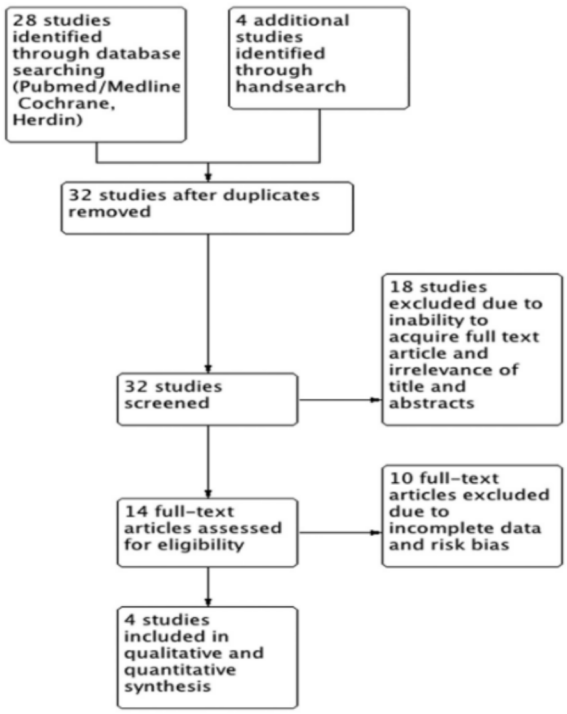

Figure 1. Study flow diagram

Table 2. Characteristics of included studies

Study	Methods	Participants	Diagnosis	Outcome
Kumar 2017	Cross sectional	306 children < 12 years with dengue fever; Jan 2013-Jun 2014	Patients categorized as dengue without and with warning signs and severe dengue; confirmed with Dengue NS1 or IgM.	Calcium level decreased among all severe dengue patients (mean = 7.3 ± 1.8), normal among non-severe dengue (mean = 8.8 ± 0.8), $p < 0.001$
Manjunath 2019	Case control	75 children 1-18 years with dengue fever; Oct 2014-Mar 2016	Patients categorized as dengue without and with warning signs and severe dengue; confirmed with Dengue NS1 antigen detection by ELISA or IgM ELISA.	Ionic calcium level decreased in 15/22 severe dengue patients (mean = 1.06; SD = 0.111), 27/53 non-severe dengue patients (mean = 1.11; SD 0.090); 7 severe dengue patients expired with a mean ionic calcium of 0.97 $p < 0.001$
Nguyen 2004	Cross sectional	107 infants < 12 months with dengue fever; Jan 1998-Mar 2002	Patients categorized as non-severe dengue (Grade I and II) and severe dengue (Grade III and IV); confirmed with Dengue IgM and IgG ELISA	Ionic calcium level decreased in 6/22 severe dengue patients (mean = 0.98)
Singh 2019	Cross sectional	100 children 1mo-18 years; Jan-Dec 2017	Patients categorized as dengue without and with warning signs and severe dengue; confirmed with Dengue NS1 antigen detection or IgM/IgG ELISA	Ionic calcium level decreased in 19/19 severe dengue patients (mean = 0.98 ± 0.05); 40/41 non-severe dengue patients (mean 1.05 ± 0.06)

selection and index test because the subjects only involved a specific age group at < 1 year old (Figures 2 & 3).

Results revealed hypocalcemia in both non-severe and severe dengue though majority was seen among the severe dengue group, especially those with fatal disease. The results are summarized in Table 3. Kumar and Singh had the highest sensitivity while Kumar and Nguyen had the highest specificity as shown in Figure 4.[7-9] The average sensitivity was 0.74 (95% CI = 0.58, 0.84) and specificity was 0.75 (95% CI = 0.67, 0.81). There is a 74% chance of hypocalcemia in a patient with severe dengue and a 75% chance that a patient with non-severe dengue will not have hypocalcemia, as shown in Figure 4. Kumar and Nguyen had the highest PPV while Kumar and Singh had the highest

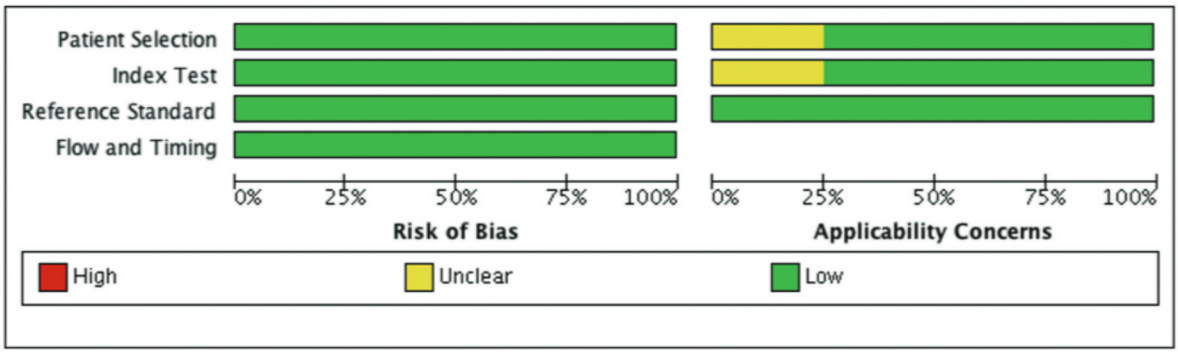

Figure 2. Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies

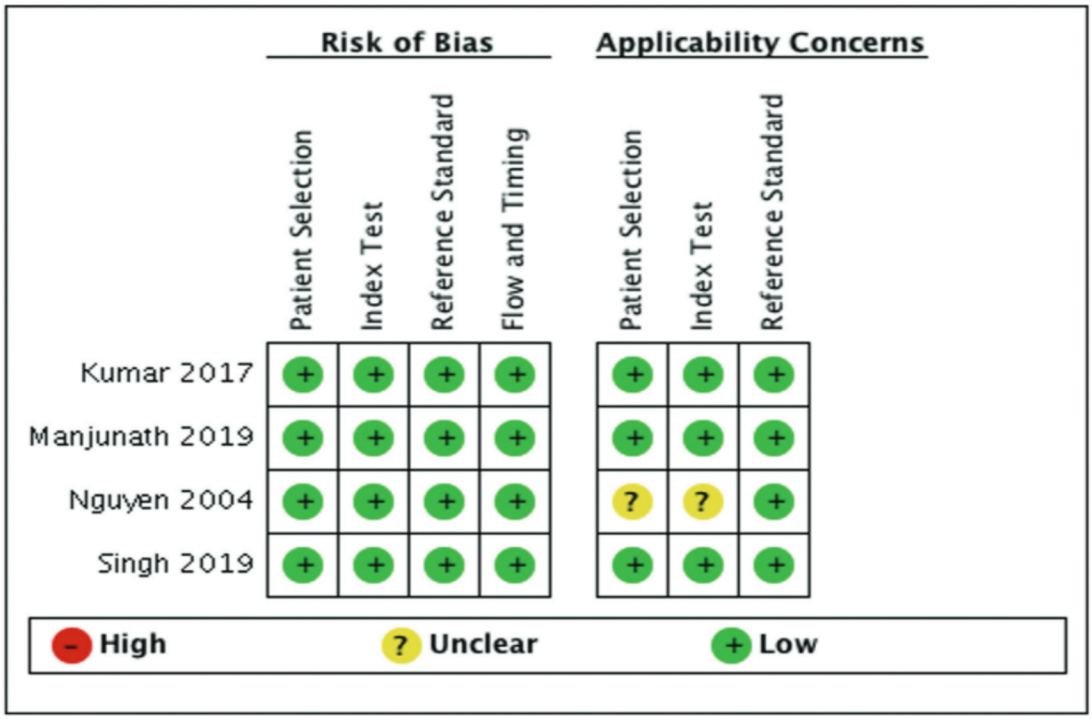

Figure 3. Risk of bias and applicability concerns summary: review authors' judgements about each domain for each included study

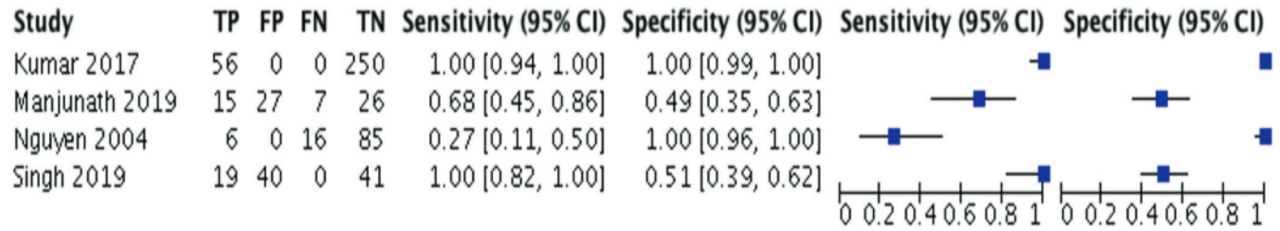

Figure 4. Forest plot of hypocalcemia in severe dengue

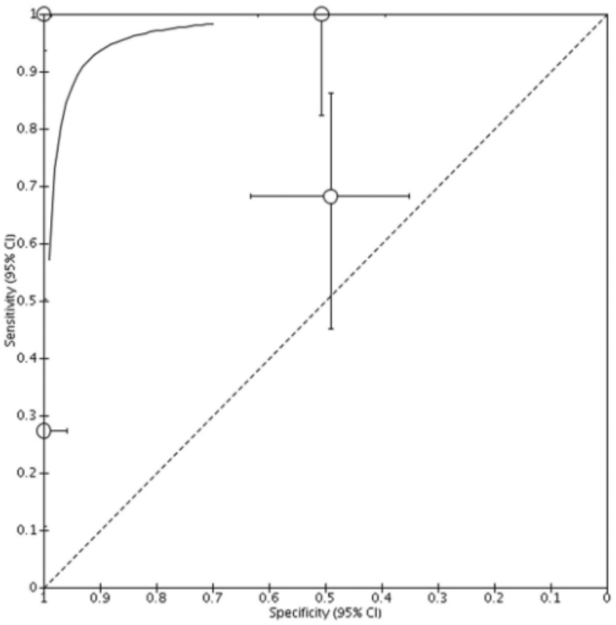

Figure 5. Summary ROC plot of hypocalcemia in severe dengue

NPV as seen in Table 4.[7-9] There is also a 67% predictive value of hypocalcemia for severe dengue. While the negative predictive value showed a 90.7% probability that when calcium levels are normal dengue will be non-severe. The summary ROC scatter plot (Figure 5) also shows greater variability in estimated sensitivity and specificity across studies. Although there was one outlier, the curve leaned to a higher sensitive and specific test.

Discussion

Based on the four included studies the average sensitivity is 0.74 and specificity is 0.75. There is a 74% chance of hypocalcemia among patients with severe dengue and a 75% chance of hypocalcemia among those with non-severe dengue. There is a 67% chance that a patient with hypocalcemia will have severe dengue and a 91% chance that a patient with no hypocalcemia will have non-severe dengue. From these percentages, the author cannot conclude that hypocalcemia is accurate in predicting a severe dengue. Thus, the author also looked into the summary receiver operator characteristic (sROC) curve. As the discriminatory power of a test increases, the sROC curve locates nearer to the top left-hand corner in ROC space towards the point where the sensitivity and specificity are both equal as seen in Figure 5.

Test accuracy may also vary according to the characteristics of the participants, setting, tests, reference standards and other methodological characteristics. This was assessed by investigating heterogeneity. The statistical power of a comparison depends on the number of studies, precision of the estimated within each study and will be lower where the characteristic is unevenly distributed across groups. In this case, such important heterogeneity or differences may be missed. This was one of the limitations of the study. The four included studies had undergone a validity appraisal, and all showed a low risk of bias. However, the review needs to include more studies to generate a more accurate result.

The included studies involved a good spectrum of the pediatric population, the serum total and ionized calcium levels were stated, and the diagnosis of dengue fever was confirmed by appropriate testing. Singh showed that the lowest mean ionized calcium (iCa) was found among severe dengue patients as compared to patients without warning signs.[9] This was similar to the findings of Habbu, Jayachandra, Azin, Manjunath, and Kumar.[5-7,10,11] A reason for

Table 3. Summary of non-severe and severe dengue patients with hypocalcemia

Study	Non-severe dengue		Severe dengue		Significance
	n*	Mean ± SD	n*	Mean ± SD	
Kumar 2017 (N = 306)	0/250	8.8 ± 0.8	56/56	7.3 ± 1.80	Hypocalcemia significant with severe dengue
Manjunath 2019 (N = 75)	Dengue without warning signs		15/22	1.06 ± 0.11	Hypocalcemia correlates with the severity of dengue illness
	6/12	1.096 ± 0.113			
	Dengue with warning signs				
	22/41	1.11 ± 0.090			
Nguyen 2004 (N = 107)	0/85	NA	6/22	0.98 ± 0.21	Not statistically significant
Singh 2019 (N = 100)	Dengue without warning signs		19/19	0.98 ± 0.05	Hypocalcemia significant with severe dengue
	4/38	1.13 ± 0.10			
	Dengue with warning signs				
	36/43	1.05 ± 0.06			

*n - number of patients who had hypocalcemia in each dengue category
Hypocalcemia defined serum ionized calcium levels < 1.1 mmol/L or total calcium levels < 7.5 mmol/L

Table 4. Predictive values and likelihood ratios of the included studies

Study	PPV	NPV	LR+	LR-	Prevalence
Kumar 2017	1	1	0	0	0.18
Manjunath 2019	0.36	0.79	1.34	0.65	0.29
Nguyen 2004	1	0.84	0	0.73	0.21
Singh 2019	0.32	1	2.03	0	0.19

PPV – positive predictive value, NPV – negative predictive value, LR+ - positive likelihood ratio, LR- - negative likelihood ratio

such finding as stated by Jayachandra is that calcium levels were lower with increasing severity of the dengue fever.[6] Several causes for low blood calcium levels have been suggested, including reduced Na^+-K^+ adenosine triphosphatase (ATPase) activity, reduced Ca^{2+}-ATPase activity, acquired parathyroid hormone deficiency, renal 1-alpha hydroxylase insufficiency, reduced dietary vitamin D intake, and reduced dietary calcium intake. Calcium appears to play a role in the induction of dengue specific T-helper cells. Dengue antigen has been shown to increase the influx of Ca^{2+} into T-cells. The proliferation of dengue-specific T-helper cells appears to be dependent on Ca^{2+} and is inhibited in the absence of Ca^{2+} and by calcium channel antagonist drugs. Despite these theories, the exact mechanism needs further investigation.

This review had limitations in terms of the inability to retrieve full text articles. These studies could have added more data to this review. Another limitation of this review is that there are few studies on pediatric patients, as majority of the studies involved adults. Lastly, the review analyses were limited only to the available data and to RevMan 5.4.1. Other analyses of diagnostic test accuracy like a hierarchical modelling can be done using other software like R or STATA or SAS but with a cost.

This review showed and summarized the significance of hypocalcemia in relation to severe dengue fever. There is a 75% sensitivity and specificity of hypocalcemia among severe dengue patients and has a 67% predictive value. However, it needs further analyses to strengthen the sensitivity and specificity of the diagnostic accuracy of hypocalcemia. This review suggests the potential benefit of determining hypocalcemia in predicting severe dengue as early recognition of patients at risk can contribute to decreasing mortality among dengue patients. However, studies as of this time of research are minimal thus the low statistical power of the review.

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